of a leucine-rich segment, a structural motif found in many transmembrane proteins. The Toll protein controls dorsal-ventral patterning in *Drosophila* embryos and activates the transcription factor Dorsal upon binding to its ligand Spätzle. (Morisato and Anderson, Cell 76, 677-688 (1994).) In adult *Drosophila*, the Toll/Dorsal signaling pathway participates in the anti-fungal immune response. (Lemaitre et al., Cell 86, 973-983 (1996).) –

B2

In the paragraphs on page 2, lines 33-39 - page 3, lines 1-11, the text has been amended as follows:

-- In one embodiment, the invention provides an isolated nucleic acid molecule comprising a DNA encoding a polypeptide having at least about 80% sequence identity, preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, most preferably at least about 95% sequence identity to (a) a DNA molecule encoding a PRO285 polypeptide having amino acid residues 30 to 836 of Fig. 1 (SEQ ID NO:1); or (b) to a DNA molecule encoding a PRO286 polypeptide having amino acid residues 27 to 825 of Fig. 3 (SEQ ID NO:3), or (c) to a DNA molecule encoding a PRO358 polypeptide having amino acids 20 to 575 of Fig. 12A-B (SEQ ID NO: 13), or (d) the complement of the DNA molecule of (a), (b), or (c). The complementary DNA molecule preferably remains stably bound to such encoding nucleic acid sequence under at least moderate, and optionally, under high stringency conditions.

B3

In a further embodiment, the isolated nucleic acid molecule comprises a polynucleotide that has at least about 90%, preferably at least about 95% sequence identity with a polynucleotide encoding a polypeptide comprising the sequence of amino acids 1 to 836 of Fig. 1 (SEQ ID NO:1); or at least about 90%, preferably at least about 95% sequence identity with a polynucleotide encoding a polypeptide comprising the sequence of amino acids 1 to 1041 of Fig. 3 (SEQ ID NO: 3); or at least about 90%, preferably at least about 95% sequence identity with a polynucleotide encoding a polypeptide comprising the sequence of amino acids 1 to 811 of Fig. 12A-B (SEQ ID NO: 13).

In the paragraph on page 4, lines 26-29, the text has been amended as follows:

B4

-- A process for producing PRO285, PRO286 and PRO358 polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of PRO285, PRO286, and PRO358, respectively, and recovering PRO285, PRO286, or PRO358 from the cell culture. --

In the paragraph on page 6, lines 28-39 - page 7, lines 1-7, the text has been amended as follows:

B5

-- Figure 6 TLR2 mediates LPS-induced signaling. **a.** 293 cells stably expressing TLR2 acquire LPS responsiveness. Either a population of stable clones expressing gD.TLR2 (293-TLR2 pop1) or a single clone of cells expressing gD.TLR2 (293-TLR2 clone 1) or control cells (293-MSCV) that were stably transfected with the expression vector alone were transiently transfected with pGL3.ELAM.tk and then

stimulated with 1 μg/ml of 055:B5 enhancer for 6 h with or without LBP in serum-free medium. Activation of the ELAM enhancer was measured as described in the Examples. Results were obtained from two independent experiments. No stimulation was observed using the control reporter plasmid that lacked the ELAM enhancer (data not sown). Expression of the reporter plasmid was equivalent in untreated cells or cells treated with LBP alone (data not shown). **b.** Western blot showing expression of epitope-tagged TLR2 in 293 cells. **c.** Time course of TLR2-dependent LPS-induced activation and translocation of NF-κB. Nuclear extracts were prepared from cells treated with 055:B5 LPS (10 μg/ml) and LBP for the indicated times (top), or cells pretreated with 1 μM cycloheximide (CHX) for 1h then stimulated with 1 μg/ml LPS for 1h in the presence of LBP in serum-free medium (bottom). **d.** Effect of mCD14 on NF-κB activation by TLR2. Vector control (193-MSCV) or 293-TLR2 pop1 cells were transfected with the reporter plasmid, and a CD14 expression vector (+mCD14) or vector control (-mCD14), respectively. After 24h, transfected cells were stimulated with 055:B5 LPS for 6h in the presence of LBP in serum-free medium. The data presented are representative from three independent experiments. --

B5 cont

In the paragraph on page 8, lines 15-28, the text has been amended as follows:

-- A "native sequence PRO285" or "native sequence PRO286" comprises a polypeptide having the same amino acid sequence as PRO285 or PRO286 derived from nature. Such native sequence Toll polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The terms "native sequence PRO285" and "native sequence PRO286" specifically encompass naturally-occurring truncated or secreted forms of the PRO285 and PRO286 polypeptides disclosed herein (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the PRO285 and PRO286 polypeptides. In one embodiment of the invention, the native sequence PRO285 is a mature or full-length native sequence PRO285 polypeptide comprising amino acids 1 to 1049 of Fig. 1 (SEQ ID NO: 1), while native sequence PRO286 is a mature or full-length native sequence PRO286 polypeptide comprising amino acids 1 to 1041 of Fig. 3 (SEQ ID NO:3). In a further embodiment, the native sequence PRO285 comprises amino acids 30-1049, or 30-836 of Fig. 1 (SEQ ID NO:1), or amino acids 27-1041, or 27-825 of Fig. 3 (SEQ ID NO:3). --

B6

In the paragraph on page 12, lines 5-21, the text has been amended as follows:

-- The term "expression vector" is used to define a vector, in which a nucleic acid encoding a Toll homologue protein herein is operably linked to control sequences capable of affecting its expression in a suitable host cell. Vectors ordinarily carry a replication site (although this is not necessary where chromosomal integration will occur). Expression vectors also include marker sequences which are capable of providing phenotypic selection in transformed cells. For example, E. coli is typically

B7

transformed using pBR322, a plasmid derived from an *E. coli* species (Bolivar, *et al.*, Gene 2: 95 [1977]). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells, whether for purposes of cloning or expression. Expression vectors also optimally will contain sequences which are useful for the control of transcription and translation, e.g., promoters and Shine-Dalgarno sequences (for prokaryotes) or promoters and enhancers (for mammalian cells). The promoters may be, but need not be, inducible; even powerful constitutive promoters such as the CMV promoter for mammalian hosts have been found to produce the LHR without host cell toxicity. While it is conceivable that expression vectors need not contain any expression control, replicative sequences or selection genes, their absence may hamper the identification of hybrid transformants and the achievement of high level hybrid immunoglobulin expression. –

B7 cont

In the paragraph on page 14, lines 19-26, the text has been amended as follows:

-- The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide fused to a "tag polypeptide." The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cor-cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues). --

B8

In the paragraph on page 14, lines 27-36, the text has been amended as follows:

-- As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and the immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. –

B9

In the paragraph on page 18, lines 15-24, the text has been amended as follows:

-- It is believed that the intracellular domain, and especially its C-terminal portion, is important for the biological function of these polypeptides. Accordingly, if the objective is to make variants which retain the biological activity of a corresponding native Toll-like protein, at least a substantial portion of these

B10

regions is retained, or the alterations, if any, involve conservative amino acid substitutions and/or insertions or amino acids which are similar in character to those present in the region where the amino acid is inserted. If, however, a substantial modification of the biological function of a native Toll receptor is required (e.g., the objective is to prepare antagonists of the respective native Toll polypeptides), the alterations involve the substitution and/or insertion of amino acids, which differ in character from the amino acid at the targeted position in the corresponding native Toll polypeptide. --

B10 cont

In the paragraph on page 18, lines 38-39 - page 19, lines 1-11, the text has been amended as follows:

-- Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e. insertions within the PRO285, PRO286 or PRO358 protein amino acid sequence) may range generally from about 1 to 10 residues, more preferably 1 to 5 residues, more preferably 1 to 3 residues. Examples of terminal insertions include the PRO285, PRO286 and PRO358 polypeptides with an N-terminal methionyl residue, an artifact of its direct expression in bacterial recombinant cell culture, and fusion of a heterologous N-terminal signal sequence to the N-terminus of the PRO285, PRO286, or PRO358 molecule to facilitate the secretion of the mature proteins from recombinant host cells. Such signal sequences will generally be obtained from, and thus homologous to, the intended host cell species. Suitable sequences include STII or Ipp for E. coli, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells. –

B11

In the paragraph on page 33, lines 3-11, the text has been amended as follows:

-- Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63]. –

B12

In the paragraph on page 37, lines 1-9, the text has been amended as follows:

-- Anti-PRO285, anti-PRO286, anti-PRO358, or anti-TLR2 antibodies also are useful for the affinity purification of these proteins from recombinant cell culture or natural sources. In this process, the antibodies against these Toll proteins are immobilized on a suitable support, such a Sephadex resin or

B13

filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the PRO285, PRO286, PRO358, or TLR2 protein which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the protein from the antibody. --

In the paragraph on page 39, lines 19-22, the text has been amended as follows:

-- Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, Virginia. --

In the paragraph on page 40, lines 19-23, the text has been amended as follows:

-- Based on a BLAST and FastA sequence alignment analysis (using the ALIGN computer program) of the full-length sequence of PRO285, it is a human analogue of the *Drosophila* Toll protein, and is homologous to the following human Toll proteins: Toll1 (DNAX# HSU88540-1, which is identical with the random sequenced full-length cDNA #HUMRSC786-1); Toll2 (DNAX# HSU88878-1); Toll3 (DNAX# HSU88879-1); and Toll4 (DNAX# HSU88880-1). –

In the paragraph on page 42, lines 31-35, the text has been amended as follows:

-- Based on a BLAST and FastA sequence alignment analysis (using the ALIGN computer program) of the full-length sequence of PRO358, it is a human analogue of the *Drosophila* Toll protein, and is homologous to the following human Toll proteins: Toll1 (DNAX# HSU88540-1, which is identical with the random sequenced full-length cDNA #HUMRSC786-1); Toll2 (DNAX# HSU88878-1); Toll3 (DNAX# HSU88879-1); and Toll4 (DNAX# HSU88880-1). –

In the paragraph on page 43, lines 6-8, the text has been amended as follows:

-- DNA comprising the coding sequence of a Toll homologue is employed as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of these particular Toll proteins) in human tissue cDNA libraries or human tissue genomic libraries. --

In the paragraph on page 44, lines 33-39 - page 45, lines 1-3, the text has been amended as follows:

-- In an alternative technique, Toll homologue DNA may be introduced into 293 cells transiently using

the dextran sulfate method described by Sompayrac et al., Proc. Natl. Acad. Sci., 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 μg pRK5-PRO(285)/(286)/(358) DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 μg/ml bovine insulin and 0.1 μg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing the corresponding expressed Toll homologue can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography. –

B18 cont

In the paragraphs on page 49, lines 18-33, the text has been amended as follows:

-- 29332 parental or stable cells (2 x 10^5 cells per well) were seeded into six-well plates, and transfected on the following day with the expression plasmids together with 0.5 μg of the luciferase reporter plasmid pGL3-ELAM.tk and 0.05 μg of the *Renilla* luciferase reported vector as an internal control. After 24 hours, cells were treated with either LPS, LBP or both LPS and LBP and reporter gene activity was measured. Data are expressed as relative luciferase activity by dividing firefly luciferase activity with that of *Renilla* luciferase. For EMSA, nuclear extracts were prepared and used in a DNA-binding reaction with a 5'-[^{32}P]-radiolabelled oligonucleotides containing a consensus NF-κB binding site (Santa Cruz Biotechnology, sc-2511). The identity of NF-κB in the complex was confirmed by supershift with antibodies to NF-κB (data not shown).

B19

RNA expression The tissue northern blot was purchased from Clontech and hybridized with a probe encompassing the extracellular domain of TLR2. Polyadenylated mRNA was isolated from 293 cells or 293-TLR2 cells and Northern blots were probed with human IL-8 cDNA fragment. TLR2 expression was determined using quantitative PCR using real time "taqman™" technology and analyzed on a Model 770 Sequence Detector (Applied Biosystems, Foster City, CA, USA) essentially as described (Luoh *et al.*, J. Mol. Endocrinol. 18, 77-85 [1997]).

In the paragraph on page 50, lines 21-28, the text has been amended as follows:

-- To examine if human TLRs might be involved in LPS-induced cell activation, we first investigated the expression of TLRs in a variety of immune tissues. One of the TLRs, TLR2, was found to be expressed in all lymphoid tissues examined with the highest expression in peripheral blood leukocytes (Figure 5a). Expression of TLR2 is enriched in monocytes/macrophages, the primary CD14-expressing and LPS-responsive cells. Interestingly, tLR2 is up-regulated upon stimulation of isolated monocytes/macrophages with LPS (Figure 5b), similar to what has been reported for CD14 (Marchant *et al.*, Eur. J. Immunol. 22, 1663-1665 [1992]; Croston *et al.*, J. Biol. Chem. 270, 16514-16517 [1995]).

B20

In the paragraph on page 51, lines 14-25, the text has been amended as follows:

-- The data presented above suggested that TLR2 might function as a signaling transducer for LPS. To examine the role of the intracellular domain ot TLR2 in mediating the LPS response, we determined if TLR2 variants with C-terminal truncations of either 13 (TLR-Δ1) or 141 amino acids (TLR2-Δ2) could regulate the ELAM reporter in transiently transfected 293 cells. We observed that both C-terminal truncation variants were defective for activation of the reporter gene although we could detect expression of these receptors at the cells surface by FACS analysis (not shown) and by Western blot (Figure 7c). The region of the intracellular domain deleted in TLR2-Δ1 bears striking similarity to a region of the IL-1R intracellular domain that is required for association with the IL-1R-associated kinase IRAK (Croston *et al., supra*) (Figure 7b). We also demonstrated that the extracellular domain (ECD) of TLR2 is required for LPS-responsiveness in that a TLR2 variant in which the ECD of TLR2 was replaced with a portion of the ECD of CD4 also failed to respond to LPS (Figure 7a and 7b). --

In the paragraph on page 52, lines 22-38, the text has been amended as follows:

-- *Drosophila* Toll and the Toll related-receptor 18 Wheeler play and important role in the induction of antimicrobial peptides in response to bacteria and fungi, respectively. Medzhitov *et al., supra.* Genetic data has implicated Spätzle as a ligand for Toll in dorsoventral patterning and has led to speculation that a homologue of Spätzle might be important for regulation of human TLRs in the immune response. Our observations that activation of TLR2 by LPS is not blocked by cycloheximide and that the extracellular domain of TLR2 is a low affinity receptor for LPS *in vitro* is consistent with a model in which TLR2 participated in LPS recognition. Our data does not exclude the possibility that other proteins (such as a Spätzle homologue) may modify the response of TLR2 to LPS. We note that while extracellular domains of TLR2 and *Drosophila* Toll both contain LRRs, they share less than 20% amino acid identity. Secondly, LRR proteins are Pattern Recognition Receptors (PRRs) for a variety of types of molecules, such as proteins, peptides, and carbohydrates. Dangl *et al.*, Cell 91, 17-24 (1997). Thirdly, the requirement for Spätzle in the *Drosophila* immune response is less clear than that of Toll. Unlike defects in Toll, Spätzle mutants induce normal levels of the antimicrobial peptides Defensin and Attacin and are only partially defective in Cecropin A expression following fungal challenge, and are not defective in activation of Dorsal in response to injury. Lemaitre *et al.*, Cell 86, 973-983 (1996); Lemaitre *et al.*, EMBO J. 14, 536-545 (1995). –

In the paragraph on page 55, lines 12-17, the text has been amended as follows:

-- In this experiment, low levels of expression were observed in the placenta and over hematopoietic cells in the mouse fetal liver. No expression was detected in either human fetal, adult or chimp lymph node